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Poster Session 2: Gene regulation, molecular and cellular biology, pharmacology, and pulmonary hypertension

Endothelin regulation by miR-218: A target in scarring

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The adult human dermis scars; scarless repair occurs in the oral cavity. Alpha-smooth muscle (a-SMA)-expressing myofibroblasts are resposible for scarring. TGFbeta1 causes myofibroblast differentiation in dermal but not gingival fibroblasts (N = 3, p&lt0.05). Gingival fibroblasts express less focal adhesion kinase, display less focal adhesion kinase phosphorylation and do not induce endothelin-1 (ET-1) in response to TGFbeta1 (N = 3, p&lt0.05). The induction of ET-1 in response to TGFbeta 1 in dermal fibroblasts does not occur in the absence of FAK or in the presence of the FAK/src inhibitor PP2 (N = 3, p&lt0.05). Addition of ET-1 to gingival fibroblasts restores the ability of TGFbeta1 to induce myofibroblast formation (N = 3, p&lt0.01). Expression profiling revealed that, compared to gingival fibroblasts, dermal fibroblasts overexpress a variety of miRNAs including miR-218. Addition of miR-218 to gingival fibroblasts results in enhanced focal adhesion kinase expression and phosphorylation, cell spreading, endothelin-1 production and in the ability of TGFbeta to induce myofibroblast formation (N-3, p&lt0.01). Knockdown of miR-218 abolishes the ability of TGFbeta1 to induce myofibroblast formation in dermal fibroblasts. These results strongly suggest that the presence or absence of ET-1 is responsible for myofibroblast formation in fibroblasts. Targeting ET-1 might be a viable approach to prevent scarring.

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miRNA-1 Regulates endothelin-1 in diabetes

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MicroRNAs-1 (miR-1) plays important roles in several biological processes. ET-1 is upregulated in chronic diabetic complications. In this study, we investigated the role of miR-1, an ET-1 targeting miRNA, in the endothelial cells (ECs) and in the organs of diabetic animals. PCR array was used to identify alteration of miR expressions in the ECs exposed to glucose. miR-1 expression was validated by TaqMan Real-Time PCR assay. Human retinal ECs (HRECs) exposed to various glucose levels with or without miR-1 mimic transfection as well as tissues from streptozotocin-induced diabetic animals after 2 months of follow-up, were examined for ET-1 mRNA and protein levels, fibronectin (FN) mRNA and miR-1 expression. Array analyses showed glucose-induced alterations of 125 miRNAs (out of 381) in ECs exposed to 25 mM glucose (HG) compared to 5 mM glucose. Fifty-one miRNAs were upregulated and 74 were downregulated. HG decreased miR-1 expression and increased ET-1 mRNA and protein levels. miR-1 mimic transfection prevented HG-induced ET-1 upregulation. Furthermore, glucose induced upregulation of FN, which is mediated in part by ET-1, was also prevented by such transfection. Diabetic animals showed decreased miR-1 expression in the retina, heart, and kidneys. In parallel, ET-1 mRNA expressions were increased in these tissues of diabetic animals compared to controls. Furthermore these tissues showed upregulation of FN. These studies indicate a novel glucose-induced molecular mechanism of tissue damage, in which miR-1 regulates ET-1 expressions in diabetes. Identifying such mechanisms may lead to potential RNA based treatment for diabetic complications. Supported by Canadian Diabetes Assn. and Heart and Stroke Foundation of Canada.

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Endothelin-1 mediates downstream profibrotic effects by transforming growth factor-beta 1 in systemic sclerosis skin fibroblasts

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Systemic sclerosis (SSc) is an autoimmune connective tissue disorder characterized by excess collagen deposition and vascular changes that affect multiple organs. Although transforming growth factorβ1 (TGF-β1) and endothelin-1 (ET-1) are known to be potent fibrotic factors in SSc, the relationship between them is not fully understood. The aim of our study was to examine the effects of TGF-β1 on the fibrogenic phenotype of SSc skin fibroblasts through ET-1 production. Human skin fibroblasts obtained from SSc patients were incubated with TGF-β1 in the presence of SIS3 (an inhibitor of Smad3 phosphorylation). In addition, the effects of ETRA, ETRB and dual ETRA/ETRB antagonist were explored. Expression of ET-1, CTGF and type I collagen was evaluated by using ELISA and real time RT-PCR. ETRA and ETRB expressions were assessed by immunohistochemistry. We found that TGF-β1 increased ET-1 mRNA and protein expression and this increase in ET-1 was suppressed by SIS3. Upregulation of COL1A1 and

CTGF by TGF-β1 was reduced by an ETRA or ETRB antagonist, and a dual ETRA/ETRB antagonist had an additive inhibitory effect. In conclusion, TGF-β1 produced ET-1 through Smad3 phosphorylation and a dual ETRA/ETRB antagonist decreased COL1A1 and CTGF mRNA levels in fibroblasts. Inhibition of ET-1 signaling may exert anti-fibrotic effects in SSc fibroblasts.

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Effect of feeding behavior on circadian regulation of endothelin expression in mouse colon epithelia

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The function, regulation and gene expression of the endothelin (ET) system in intestine are not well understood. We investigated the dependence on feeding schedule and biological clock of the regulation of ET-1 gene expression in mouse colon. Mice were fed freely, fasted for 48 h, and re-fed after fasting. Gene expression was analyzed by real-time RT-PCR. ET-1 gene expression was highest in colon compared with other tissues examined in fasted mice. Fasting increased the amplitude, while maintaining the rhythmicity, of ET-1 gene expression in epithelial colonic tissue. Re-feeding, however, decreased gene expression and suppressed rhythmic oscillation, even though the rhythmicity of Per-1 and Per-2 gene expression remained unchanged. Furthermore, the decrease in ET-1 gene expression induced by re-feeding was blocked by pre-treatment with hexamethonium and atropine. The daily change in ET-1 gene expression and peptide production in colon epithelia, which depends on feeding schedule via autonomic nervous system, is synchronized with peripheral circadian oscillators under conditions of free feeding and fasting but not re-feeding. ET-1 plays important physiological roles, which is dependent on feeding behavior.

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cDNA cloning and sequence analysis of preproendothelin from barfin Flounder (*Verasper moseri*)

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The presence of endothelin (ET)-like immunoreactivity and the cardiovascular effects of mammalian ET-1 in fish have been reported. To identify ET-related peptides in fish, we screened the cDNA library of the barfin flounder (*Verasper moseri*) intestine by means of rapid amplification of cDNA ends, and we cloned cDNAs encoding an ET-related peptide. The ET-related sequence of 21 amino acids is similar to the trout ET-1 peptide recently purified from kidney specimens of *Oncorhynchus mykiss*. The deduced amino acid sequence of pre- proET-1 (PPET-1) comprises 244 amino acids, including a putative signal sequence and mature ET-1, as well as big ET-1 and ET-1-like sequences. This precursor, the first reported PPET-1 sequence, has low homology with the sequences of human, mouse, frog (*Xenopus laevis*), and zebrafish (*Danio rerio*) PPET-1.

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Shark endothelin: cDNA cloning, sequence and evolutional analysis

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Endothelin (ET)-related receptors homologous to mammalian receptors have been cloned from fish, indicating that ET-related ligands may be present in lower species. Here we cloned cDNAs encoding preproendothelin (PPET) from the Shark intestinal cDNA library. Shark ET cDNAs encode 200 amino acids, including a 20-amino-acid putative signal sequence, as well as mature ET, big ET, and ET-like sequences. This sequences together with other published PPET sequences were used to analyze the phylogenetic relationship among all ET family genes.

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Molecular cloning and sequence analysis of preproendothelin from medaka, *Oryzias latipes*

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The presence of endothelin (ET)-like immunoreactivity and the cardiovascular effects of mammalian ET-1 in fish have been reported. To identify ET-related peptides in fish, we screened the cDNA library of the medaka (*Oryzias latipes*) intestine by means of rapid amplification of cDNA ends, and we cloned cDNAs encoding an ET-related peptide. The medaka ET-related sequence of 21 amino acids is similar to the trout ET-1 peptide recently purified from kidney specimens of *Oncorhynchus mykiss*. The deduced amino acid sequence of pre-proET-1 (PPET-1) comprises 200 amino acids, including a putative signal sequence and mature ET-1, as well as big ET-1 and ET-1-like sequences. This precursor, the first reported PPET-1 sequence, has low homology with the sequences of human, mouse, frog (*Xenopus laevis*), and zebrafish (*Danio rerio*) PPET-1.

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